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PII: S0040-4020(13)01756-0

DOI: 10.1016/j.tet.2013.11.054

Reference: TET 25031

To appear in: *Tetrahedron*

Received Date: 12 August 2013

Revised Date: 1 November 2013

Accepted Date: 18 November 2013

Please cite this article as: Brzozowski M, O'Brien NJ, Wilson DJD, Abbott BM, Synthesis of substituted 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential PDK1 inhibitors, *Tetrahedron* (2013), doi: 10.1016/j.tet.2013.11.054.

Tetrahedron
journal homepage: www.elsevier.com

# Synthesis of substituted 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential PDK1 inhibitors

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ARTICLE INFO

*Article history:*
Received
Received in revised form
Accepted
Available online

*Keywords:*
3-Phosphoinositide-dependent kinase 1 (PDK1)
4-(1*H*-indol-6-yl)-1*H*-indazole
Inhibitor
Suzuki cross-coupling

ABSTRACT

The development of a preparative route to a series of novel 4-(1*H*-indol-6-yl)-1*H*-indazole compounds as potential PDK1 inhibitors is described. The synthetic strategy centres on the late stage Suzuki cross-coupling of *N*-unprotected indazole and indole fragments. The use of a monoligated palladium catalyst system was found to be highly beneficial in the cross-coupling reaction. The indazole and indole fragments were constructed by diazotisation/cyclisation and $S_NAr$/reductive cyclisation sequences, respectively.



## 1. Introduction

3-Phosphoinositide-dependent kinase 1 (PDK1) is an integral member of the PI3K/Akt signalling pathway. Its activity is modulated by phosphatidylinositol 3-kinase (PI3K) through the regulation of phosphatidylinositol 3,4,5-triphosphate ($PtdInsP_3$). The PI3K/Akt signalling pathway is responsible for the regulation of cellular processes such as growth, metabolism, proliferation and survival.[1] PDK1 is known to activate at least 23 different members of this pathway including Akt,[2,3] PKC isoforms,[4] p70 S6K,[5] p90 SGK[6] and RSK.[7]

Many human cancers exhibit mutations that result in high levels of the secondary messenger, $PtdInsP_3$, leading to constitutive activation of members of the PI3K/Akt pathway.[8,9] Inappropriate activation of this pathway promotes tumour progression and angiogenesis while inhibiting apoptosis.[10] Experimental evidence has shown that inhibitors of this pathway are able to sensitise cells to apoptosis and reduce tumour formation.[11] Given its regulatory role in the PI3K/Akt pathway, PDK1 provides an attractive target for the development of oncology therapeutics.*

We aimed to develop novel scaffolds that bound to the PDK1 hinge region and allowed for straightforward incorporation of additional functionality to target various pockets within the ATP binding site. Using a molecular modelling-guided design approach, we identified that a 4-(1*H*-indol-6-yl)-1*H*-indazole core possessed the correct geometry to fulfill both of these needs (Figure 1). The heterocyclic ring nitrogen atoms form a hydrogen bond donor/acceptor/donor motif that provides key interactions with the backbone residues, Ser89 and Ala91. Functionalisation of the indole ring at the 2- and 4-positions was anticipated to provide access to the solvent exposed entrance regions ($E_0$ and $E_1$) and the ribose pocket (R), respectively. Appending functionality at the 6-position of the indazole ring would allow the catalytic residues in the phosphate pocket (P) and also the hydrophobic pocket (BP-I) to be targeted.

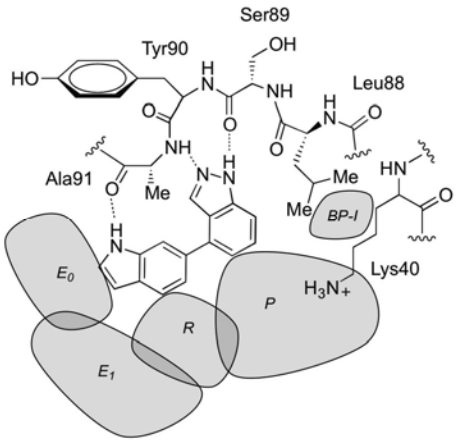


**Figure 1.** Schematic illustrating how the 4-(1*H*-indol-6-yl)-1*H*-indazole scaffold interacts with the hinge region of PDK1 and displays potential to access various pockets within the binding site.

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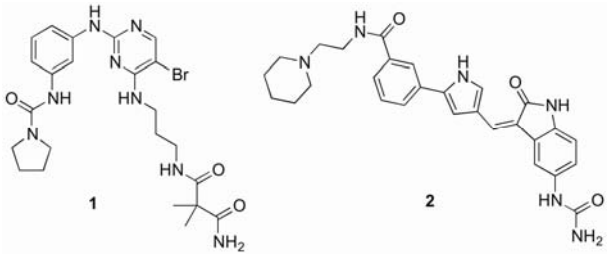


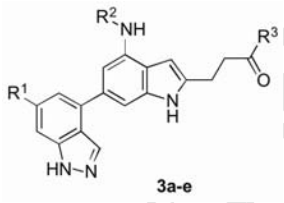
**Figure 2.** PDK1 inhibitors developed by Berlex Biosciences.

The side chain functionalities of the designed compounds were based on fragments of known inhibitors that had been shown to interact with the targeted pockets. Compounds **1** and **2** (Figure 2) are potent PDK1 inhibitors developed by Berlex Biosciences,[12,13] with X-ray crystal structures available of both compounds in complex with PDK1 (PDB codes: 1Z5M and 2PE2). Both of these structures were utilised in the design of the first 4-(1*H*-indol-6-yl)-1*H*-indazole target structure, **3a** (Table 1).

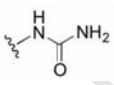
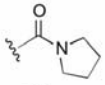
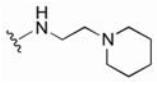
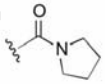
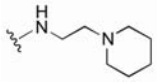
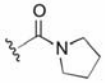
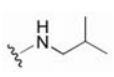
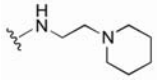
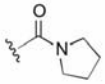
Compound **1** contains a pyrrolidine urea moiety that binds to the ribose pocket of PDK1 and this side chain was incorporated at the indole 4-position of **3a** ($R^2$ side chain). Compound **2** displays a primary urea group that interacts with catalytic residues in the phosphate pocket and a *N*-(2-(piperidin-1-yl)ethyl)amide group that binds in the $E_0$ region. These two side chain groups were integrated into **3a** at the indazole 6-position ($R^1$) and the indole 2-position ($R^3$).

We identified a small set of target structures (**3a-c**, Table 1) to be synthesised and subject to biological evaluation. Herein, we report the development of a synthetic route to this novel class of heterocyclic compounds. The resulting synthesis is modular in nature, with derivatisation of late stage intermediates giving simple access to all target structures.

**Table 1.** 4-(1*H*-Indol-6-yl)-1*H*-indazoles designed as potential PDK1 inhibitors.



**3a-e**

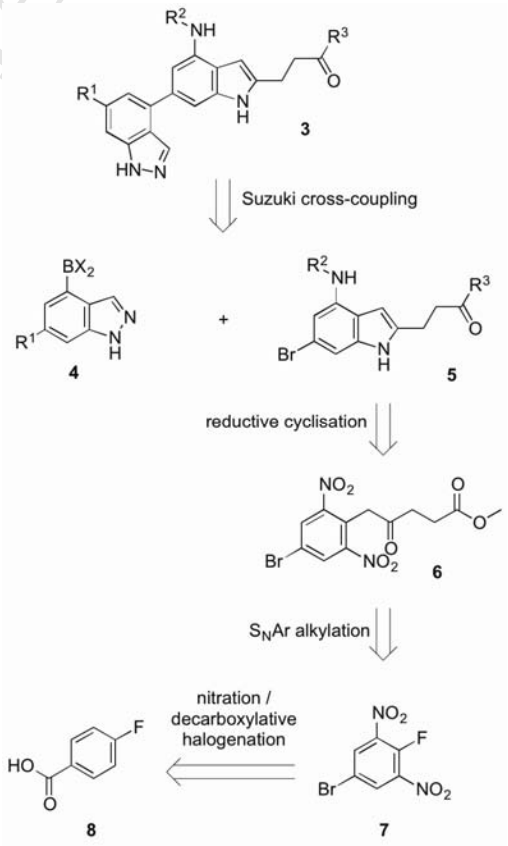
| Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **3a** |  |  |  |
| **3b** |  |  |  |
| **3c** |  |  |  |
| **3d** |  |  |  |
| **3e** |  |  |  |

## 2. Results and discussion

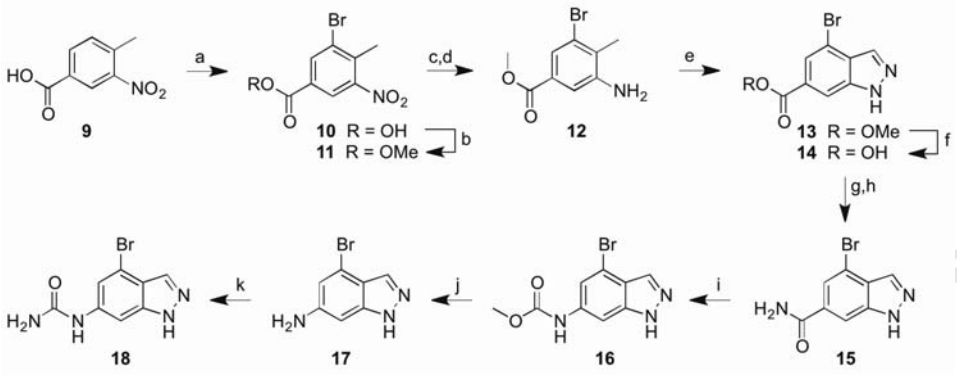
The synthetic strategy was centred on synthesising the indazole and indole fragments separately and then forming the biaryl linkage by a late-stage Suzuki cross-coupling reaction (Scheme 1). The indole intermediate **5** was proposed to be accessible by a $S_NAr$/reductive cyclisation sequence of the activated aryl fluoride **7**. Nitration and decarboxylative halogenation of the commercially available starting material **8** was anticipated as a means of providing the alkylation precursor.

Preparation of the requisite indazole fragments largely followed that of similar 4,6-disubstituted indazoles reported in the literature[14,15] (Scheme 2). *Ortho*-toluidine **12** was synthesised from 4-methyl-3-nitrobenzoic acid **9** according to literature methods.[16,17] Diazotisation and subsequent intramolecular cyclisation of **12** then furnished the indazole **13**. A hypervalent iodine mediated Hofmann rearrangement was used to complete the conversion of the acid **14** to the corresponding homologated amine **17**. Treatment of **17** with isocyanic acid, produced *in situ*, gave the primary urea indazole fragment **18**.

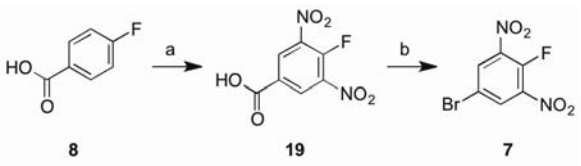
4-Fluoro-3,5-dinitrobenzoic acid (**19**) was prepared in good yield by the method of Nielsen[18] (Scheme 3). A photoassisted Hunsdiecker reaction was then employed to effect the decarboxylative halogenation of acid **19**. The method of Meyers and Fleming[19] provided the desired aryl bromide **7** in a moderate yield of 59% (Table 2, entry 1). Efforts to substitute carbon tetrachloride with less hazardous solvents led to unacceptable reductions in yield or decomposition (Table 2, entries 2-4).




**Scheme 1.** Retrosynthetic approach to the novel 4-(1*H*-indol-6-yl)-1*H*-indazole series.




**Scheme 2.** Reagents and conditions: (a) 1,3-dibromo-5,5-dimethylhydantion, $H_2SO_4$, 98%; (b) cat. $H_2SO_4$, 4 Å MS, MeOH, reflux, 90%; (c) $SnCl_2$ (5 eq.), EtOH, reflux, 30 min; (d) 15% aq. NaOH, $CH_2Cl_2$, 80%; (e) $NaNO_2$, 90% aq. AcOH, 72%; (f) 15% aq. NaOH, EtOH, 83%; (g) $SOCl_2$, reflux; (h) $NH_4OH$, THF, 0 °C, 87%; (i) $PhI(OAc)_2$, KOH, MeOH, 0 °C, 51%; (j) KOH (10 eq.), 80% aq. MeOH, reflux, 2 d, 72%; (k) NaOCN (2 eq.), 80% aq. AcOH, 0 °C, 3 h, 74%.




**Scheme 3.** Reagents and conditions: (a) fuming $HNO_3$, fuming $H_2SO_4$, 100 °C, 3 h, 68%; (b) See Table 2.

Attempts to replace mercury(II) oxide with (diacetoxyiodo)benzene, and bromine with iodine, were all unsuccessful with only traces of product observed at best (Table 2, entries 5-7). Upon reverting to the originally successful conditions, the product yield was found to diminish significantly when the reaction was scaled up. Sensitivity to water formed during the reaction was identified as a plausible cause of this difficulty. For substrates with poor solubility, the decarboxylation takes place at the solvent interface, where the intermediate acyl hypohalite is prone to attack by water as it is formed.[20] A two-step procedure, involving Dean-Stark trapping of water prior to halogen addition was found to be highly advantageous (Table 2, entry 10). Further investigation revealed the reaction to be concentration dependent, with poor results obtained at concentrations higher than 0.2 M (Table 2, entries 9 and 11).

Treatment of the activated aryl fluoride **7** with the enolate of 1-*tert*-butyl 6-methyl 3-oxohexanedioate[21,22] gave the nucleophilic aromatic substitution product **20** (Scheme 4), which existed exclusively in the (*Z*)-enol form due to a strong intramolecular hydrogen bond. The enol **20** was then smoothly decarboxylated in excellent yield to provide the ketone **6**. Catalytic hydrogenation with platinum(IV) oxide gave rapid reductive cyclisation of ketone **6** to the *N*-hydroxyindole **21** in a 62% isolated yield. Careful reaction monitoring was required to avoid hydrodehalogenation. The aminoindole **22** was obtained by further reduction of **21** with 10 equivalents of zinc powder in a 1:3 mixture of acetic acid and methanol at room temperature.

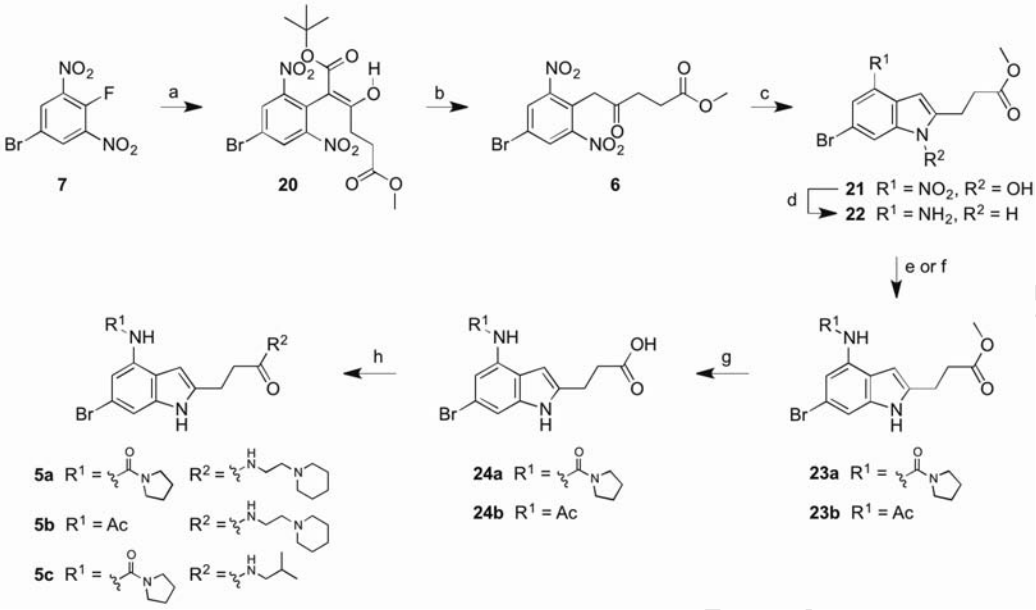
**Table 2.** Optimisation of decarboxylative halogenation reaction conditions.

| Entry | Conditions[a] | Solvent[b] | Scale (g) | Concentration (M) | Yield % |
|---|---|---|---|---|---|
| 1 | Red HgO, $Br_2$, 150 W bulb, reflux, 3 h | $CCl_4$ | 0.2 | 0.08 | 59 |
| 2 | Red HgO, $Br_2$, 150 W bulb, reflux, 3 h | $CHCl_3$ | 0.2 | 0.08 | 38 |
| 3 | Red HgO, $Br_2$, 150 W bulb, reflux, 3 h | $CH_2Cl_2$ | 0.2 | 0.08 | 16 |
| 4 | Red HgO, $Br_2$, 150 W bulb, reflux, 4 h | PhCl | 0.2 | 0.10 | 0 (dec.) |
| 5 | $PhI(OAc)_2$, $I_2$, 150 W bulb, reflux, 3 h | $CCl_4$ | 0.1 | 0.04 | trace[c] |
| 6 | $PhI(OAc)_2$, $Br_2$, 150 W bulb, reflux, 3 h | $CCl_4$ | 0.1 | 0.04 | 0 |
| 7 | Red HgO, $I_2$, 150 W bulb, reflux, 4 h | $CCl_4$ | 0.1 | 0.04 | 0[c] |
| 8 | Red HgO, $Br_2$, 150 W bulb, reflux, 4 h | $CCl_4$ | 2.0 | 0.15 | 52 |
| 9 | Red HgO, $Br_2$, 150 W bulb, reflux, 5 h | $CCl_4$ | 8.0 | 1.2 | 34 |
| 10 | 1. red HgO, reverse phase Dean-Stark trap, reflux, 1.5 h; 2. $Br_2$, 150 W bulb, reflux, 3 h | $CCl_4$ | 2.0 | 0.2 | 72 |
| 11 | 1. red HgO, reverse phase Dean-Stark trap, reflux, 7 h; 2. $Br_2$, 150 W bulb, reflux, 5 d | $CCl_4$ | 10.0 | 1.5 | 25 |
| 12 | 1. red HgO, reverse phase Dean-Stark trap, reflux, 2 d; 2. $Br_2$, 150 W bulb, reflux, 27 h | $CCl_4$ | 15.0 | 0.2 | 56 |

[a]All reactions conducted under argon using oven-dried glassware. 1.5 eq. each of oxidising agent and halogen used in all cases.

[b]All solvents were distilled prior to use and stored over 4 Å molecular sieves.

[c]Aryl iodide product.

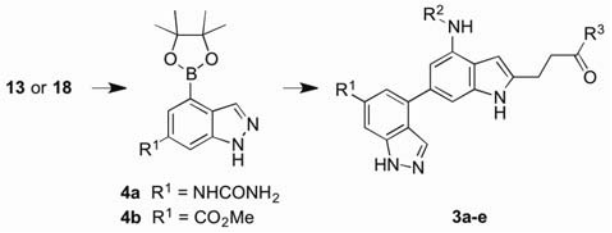



**Scheme 4.** Reagents and conditions: (a) NaH, 1-*tert*-butyl 6-methyl 3-oxohexanedioate, THF, 0 °C → rt, 62%; (b) 10% HCl in MeOH, reflux, 93%; (c) $H_2$ (1 atm.), $PtO_2$ (2.5% w/w), MeOH, 15-20 min, 62%; (d) Zn (10 eq.), 1:3 AcOH/MeOH, 24 h, 78%; (e) Triphosgene, $C_5H_5N$, $CH_2Cl_2$, 0 °C, 2.5 h, then pyrrolidine, 68%; (f) $Ac_2O$, $Et_3N$, $CH_2Cl_2$, 79%; (g) 15% aq. NaOH, MeOH, 82-88%; (h) EDAC·HCl, HOBt·$H_2O$, $CH_2Cl_2$, 0.5 h, then 2-(piperidin-1-yl)ethanamine or 2-methylpropan-1-amine, 57-81%.

Elaboration of the key indole intermediate **22** at the 2- and 4-positions provided facile access to all of the required indole cross-coupling fragments. *N*,*N*-disubstituted urea **23a** was prepared in a 68% yield by reacting the amine **22** with triphosgene and then pyrrolidine. A slow, inverse addition of the amine to a triphosgene solution was required to avoid the formation of a symmetrical dimer as the major product. Acetylation of **22** under standard conditions yielded the *N*-acetylindole **23b**. Ester hydrolysis with aqueous sodium hydroxide afforded the free acids **24a** and **24b** in high yields. Diimide mediated coupling of the acids with the appropriate amine then furnished the carboxamide fragments **5a-c**.

The 4-(1*H*-indol-6-yl)-1*H*-indazoles **3a-e** were synthesised by Miyaura borolation of indazole bromides **18** and **13**, followed by Suzuki cross-coupling of the corresponding indazole boronate esters **4a** and **4b** (Table 3). Miyaura borolation conditions[23] gave full conversion of the primary urea **18** to the boronate ester **4a**, but this compound could not be successfully purified by flash chromatography or crystallisation due to poor solubility. Attempts to employ impure **4a** in the cross-coupling step were unsuccessful and thus a one-pot procedure was developed to avoid isolation of the boronate intermediate (Table 3, entry 1). This one-pot procedure provided the desired biaryl **3a**, albeit in a poor yield of 24% over two steps, with multiple side products observed by TLC. Fragments **4b** and **5a** gave no cross-coupled product when treated with $PdCl_2$(dppf) and caesium carbonate in a refluxing 4:1 mixture of 1,4-dioxane and water (Table 3, entry 2). MS analysis indicated that significant hydrolysis of the methyl ester **4b** was observed under these reaction conditions. Caesium carbonate is known to facilitate ester cleavage[24,25] and it is plausible that the carboxylate formed by the competing hydrolysis reaction led to inhibition of the already sluggish cross-coupling reaction by catalyst deactivation.

**Table 3.** Synthesis of 4-(1*H*-indol-6-yl)-1*H*-indazoles by sequential Miyaura borolation and Suzuki cross-coupling of indazole bromides.




| Entry | Substrate | Conditions[a] | Bromide | Product | Yield % |
|---|---|---|---|---|---|
| 1 | **18** | A | **5a** | **3a** | 24 |
| 2 | **13** | B, C | **5a** | **3b** | 77, 0 |
| 3 | **13** | B, D | **5a** | **3b** | 77, 56[b] |
| 4 | **13** | B, D | **5c** | **3c** | 77, 70[b] |
| 5 | **13** | B, D | **5b** | **3d** | 77, 67[b] |
| 6 | **13** | B, D | **23a** | **3e** | 77, 44[b] |

[a]Conditions: (A) $PdCl_2$(dppf) (5 mol %), bis(pinacolato)diboron (1.4 eq.), KOAc, MeOH, reflux, 16 h, then indole bromide (1.3 eq.), $PdCl_2$(dppf) (5 mol %), $Cs_2CO_3$, 4:1 1,4-dioxane/water, reflux, 4 h; (B) $PdCl_2$(dppf) (3 mol %), bis(pinacolato)diboron (1.4 eq.), KOAc, MeOH, reflux, 16 h; (C) $PdCl_2$(dppf) (5 mol %), indole bromide (1.3 eq.), $Cs_2CO_3$, 4:1 1,4dioxane/water, reflux, 2 h. (D) $Pd_2(dba)_3$ (5 mol %), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (10 mol %), indole bromide (0.6 eq.), $Na_2CO_3$, 4:1 1,4-dioxane/water, reflux, 2-4 h.

[b]Yield based on bromide coupling partner.

For the synthesis of biaryls **3b-e**, more active catalyst systems were sought to improve the efficiency of the cross-coupling reaction and the milder base, sodium carbonate, was employed to avoid the competing ester hydrolysis. A dialkylbiarylphosphine palladium catalyst system developed by Buchwald and co-workers[26-28] gave smooth cross-coupling of boronate ester **4b** with the indole bromides **5a-c** and **23a** (Table 3, entries 3-6). This catalyst system provided greatly enhanced coupling efficiency, with only a single product observed by TLC in all cases. This increased efficiency is attributed to the formation of a highly active monoligated palladium complex,[29] which rapidly undergoes oxidative insertion with traditionally sluggish substrates such as the *N*-unprotected heteroaryl halides studied in this work.

## 3. Conclusion

In summary, we have developed an expedient route for the preparation of novel 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential inhibitors of PDK1. A $S_NAr$/reductive cyclization sequence gave access to a variety of 6-bromo-2,4-disubstituted-1*H*-indoles, which were then cross-coupled with 1*H*-indazol-4-yl-boronate esters. The use of a highly active monoligated palladium catalyst system was found to greatly improve the efficiency of the late-stage Suzuki cross-coupling. Preliminary biological screening of these compounds is currently underway and will be reported shortly.

## 4. Experimental section

### 4.1. General methods

Melting points were measured on a Reichert Thermopan microscope hot stage apparatus and are uncorrected. All glassware used in moisture sensitive reactions was oven dried and then cooled under argon prior to use. The standard workup procedure for an organic extract comprised washing with brine, drying over anhydrous magnesium sulfate and filtration, followed by concentration under reduced pressure to afford the crude product. Analytical Thin Layer Chromatography (TLC) was performed on Merck kieselgel 60 $F_{254}$ plates aluminium backed plates and visualised using a 254 nM UV lamp or by staining with ninhydrin stain consisting of ninhydrin (0.2 g), acetic acid (0.5 mL), and water (4.5 mL) in *n*-butanol (100 mL). Flash chromatography was performed on silica gel (Davisil® LC60Å 40-63 micron) according to the method of Still et al.[30] Flash chromatography eluent systems containing dichloromethane saturated with ammonia were freshly prepared as follows: Dichloromethane (400 mL) and ammonium hydroxide (30%, 40 mL) were shaken in a separating funnel, and the cloudy dichloromethane layer was separated. Upon addition of the required volume of methanol, the solution became homogenous and was used immediately. NMR spectra were recorded on a Bruker AV-500 at 500.19 MHz for $^1$H nuclei and at 125.78 MHz for $^{13}$C nuclei. Chemical shifts are reported as δ values in parts per million (ppm). In reporting spectral data the following abbreviations have been used: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants (*J*) are reported to the nearest 0.5 Hz. Low-resolution electrospray ionisation (ESI) mass spectra were recorded on a Bruker Daltronics Esquire 6000 Ion Trap mass spectrometer in methanol (0.1% formic acid) at 40 eV cone voltage. High-resolution electrospray ionisation mass spectra were recorded on an Agilent 6224 TOF LC/MS mass spectrometer coupled to an Agilent 1290 Infinity and were reference mass corrected via a dual-spray electrospray ionisation source. Preparative reverse phase high performance liquid chromatography (HPLC) was performed on a Beckman system (125 Solvent Module and 166 Detector) fitted with a Phenomenex® Jupiter C18 300 Å column (250 mm × 10.0 mm, 10 µm) at a flow rate of 5 mL/min, monitored at 220 nm.

### 4.2. Synthesis

#### *4.2.1. Methyl 4-bromo-1H-indazole-6-carboxylate (**13**)*

To a stirred solution of amine **12** (4.50 g, 18.4 mmol) in acetic acid (80 mL), was added a solution of sodium nitrite (1.39 g, 20.3 mmol) in water (8 mL). The mixture was stirred overnight and then concentrated under reduced pressure. The residue was diluted with saturated sodium hydrogen carbonate (100 mL), extracted with ethyl acetate (3 × 30 mL) and the combined organic extracts were subject to standard workup. The crude product was recrystallised from toluene and the mother liquors were purified by flash chromatography (15% EtOAc/hexanes) to afford the indazole **13** (3.38 g, 72%) as a pale orange solid; $R_f$ (15% EtOAc/hexanes) 0.25; mp 173-174 ºC; $\delta_H$ (DMSO-$d_6$): 13.83 (1H, br s), 8.16 (2H, s), 7.78 (1H, d, *J* 1.0 Hz), 3.90 (3H, s); $\delta_C$ (DMSO-$d_6$): 165.3, 139.7, 133.4, 128.4, 125.9, 122.6, 113.2, 111.9, 52.5; *m/z* (ESI): 254.9 (M[$^{79}$Br]H$^+$), 256.9 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 254.9765. $C_9H_8BrN_2O_2$ requires 254.9769.

#### *4.2.2. 4-Bromo-1H-indazole-6-carboxylic acid (**14**)*

A mixture of ester **13** (3.38 g, 13.3 mmol) and 15% aqueous sodium hydroxide (15 mL) in ethanol (30 mL) was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was diluted with water, acidified to pH 4 with 1 M hydrochloric acid, and the resulting orange precipitate was collected by filtration and dried *in vacuo* to give the acid **14** (2.64 g, 83%) as a pale orange solid; $R_f$ (10% MeOH/$CH_2Cl_2$) 0.10; mp 294 - 296 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.78 (1H, br s), 13.3 (1H, br s). 8.15 (2H, s), 7.80 (1H, d, *J* 0.5 Hz); $\delta_C$ (DMSO-$d_6$): 166.4, 139.9, 133.4, 129.9, 125.7, 123.0, 113.0, 111.8; *m/z* (ESI): 240.9 (M[$^{79}$Br]H$^+$), 242.9 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 240.9607. $C_8H_6BrN_2O_2$ requires 240.9613.

#### *4.2.3. 4-Bromo-1H-indazole-6-carboxamide (**15**)*

A stirred suspension of acid **14** (1.46 g, 6.0 mmol) and thionyl chloride (10 mL) was heated to reflux. After 1 h, the mixture became homogeneous and the solution was concentrated under reduced pressure. Dry toluene (30 mL) was added and the mixture was evaporated to dryness to remove trace thionyl chloride. The residue was suspended in dry tetrahydrofuran (50 mL), cooled to 0 ºC, and 30% ammonium hydroxide (20 mL) was added dropwise. After being stirred overnight, the mixture was diluted with water (100 mL), and the resulting precipitate was collected by filtration and dried *in vacuo* to afford the carboxamide **15** (1.26 g, 87%) as a yellow solid; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.25; mp 262-263 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.78 (1H, br s), 8.20 (1H, br s), 8.11 (1H, s), 8.10 (1H, s), 7.83 (1H, d, *J* 1.0 Hz), 7.55 (1H, br s); $\delta_C$ (DMSO-$d_6$): 166.8, 140.0, 133.5, 133.3, 125.0, 122.2, 112.9, 109.7; *m/z* (ESI): 240.0 (M[$^{79}$Br]H$^+$), 242.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 239.9766. $C_8H_7BrN_3O$ requires 239.9772.

#### *4.2.4. Methyl (4-bromo-1H-indazol-6-yl)carbamate (**16**)*

Carboxamide **15** (750 mg, 3.12 mmol) was added to a stirred solution of potassium hydroxide (438 mg, 7.80 mmol) in methanol (50 mL). Once the reaction mixture was mostly homogenous, it was cooled to 0 ºC and (diacetoxyiodo)benzene (1.01 g, 3.12 mmol) was added in a single portion. After 3 h, the reaction mixture was concentrated under reduced pressure, diluted with saturated ammonium chloride (100 mL) and the resulting precipitate collected by filtration. The precipitate was

suspended in boiling methanol (30 mL) and filtered whilst hot to remove insoluble impurities. The filtrate was evaporated under reduced pressure to give the crude product, which was purified by flash chromatography (3% MeOH/$CH_2Cl_2$) to give the carbamate **16** (429 mg, 51%) as a fawn coloured crystalline solid; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.41; mp 198-204 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.21 (1H, br s), 9.93 (1H, br s), 7.90 (1H, s), 7.83 (1H, s), 7.38 (1H, d, *J* 1.5 Hz), 3.70 (3H, s); $\delta_C$ (DMSO-$d_6$): 154.0, 141.0, 138.5, 133.0, 119.7, 115.8, 112.9, 96.8, 51.9; *m/z* (ESI): 270.0 (M[$^{79}$Br]H$^+$), 271.9 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 269.9872. $C_9H_9BrN_3O_2$ requires 269.9878.

*4.2.5. 4-Bromo-1H-indazol-6-amine (**17**)*

A stirred solution of carbamate **16** (338 mg, 1.60 mmol) and potassium hydroxide (896 mg, 16.0 mmol) in a 4:1 mixture of methanol/water (50 mL) was heated to reflux. After 2 d, the reaction mixture was concentrated under reduced pressure, diluted with saturated ammonium chloride (100 mL) and extracted with ethyl acetate (6 × 20 mL). The combined organic fractions were treated according to standard workup to give the crude product, which was purified by flash chromatography (50% EtOAc/hexanes) to afford the amine **17** (244 mg, 72%) as a dark orange crystalline solid; $R_f$ (50% EtOAc/hexanes) 0.23; mp 146-147 ºC (dec); $\delta_H$ (DMSO-$d_6$): 12.57 (1H, br s), 7.66 (1H, s), 6.73 (1H, d, *J* 1.5 Hz), 6.48 (1H, t, *J* 1.0 Hz), 5.45 (2H, br s); $\delta_C$ (DMSO-$d_6$): 148.8, 142.5, 132.8, 116.4, 114.5, 113.0, 89.5; *m/z* (ESI): 211.9 (M[$^{79}$Br]H$^+$), 213.9 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 211.9816. $C_7H_7BrN_3$ requires 211.9823.

*4.2.6. 1-(4-Bromo-1H-indazol-6-yl)urea (**18**)*

To a stirred solution of amine **17** (500 mg, 2.40 mmol) in a 5:1 mixture of acetic acid/water (12 mL) at 0 ºC, was added sodium cyanate (307 mg, 4.70 mmol). After 1 h, the reaction was diluted with water (50 mL) and the precipitate collected by filtration. The filtrate was concentrated to a small volume (5 mL) and cooled on ice to yield more of the precipitate. The combined solids were reslurried in warm *n*-butanol, filtered and dried *in vacuo* to give the urea **18** (446 mg, 74%) as an off-white powder; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.11; mp >300 ºC; $\delta_H$ (DMSO-$d_6$): 13.04 (1H, br s), 8.82 (1H, br s), 7.85 (1H, s), 7.81 (1H, s), 7.26 (1H, s), 5.95 (2H, br s); $\delta_H$ (DMSO-$d_6$): 155.9, 141.3, 139.9, 132.8, 119.1, 115.9, 112.6, 96.1; *m/z* (ESI): 254.9 (M[$^{79}$Br]H$^+$), 256.9 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 254.9874. $C_8H_9BrN_4O$ requires 254.9881.

*4.2.7. 5-Bromo-2-fluoro-1,3-dinitrobenzene (**7**)*

Acid **19** (15.0 g, 65.3 mmol) and red mercury(II) oxide (21.2 g, 98.0 mmol) were suspended in dry carbon tetrachloride (300 mL). The flask was equipped with a reverse phase Dean-Stark trap, and the heterogeneous mixture was heated to reflux while being stirred vigorously. After 2 d, approximately 1 mL of water was observed in the Dean-Stark trap. A solution of bromine (5.0 mL, 98.0 mmol) in carbon tetrachloride (1 M) was added dropwise while the reaction flask was irradiated with a 150W bulb. After 27 h, the reaction was cooled to room temperature and saturated sodium hydrogen carbonate (100 mL) was added. The two-phase mixture was filtered through Celite®, and the filtrate was extracted with carbon tetrachloride (3 × 50 mL). The combined organic fractions were treated according to standard workup to provide the bromide **7** (9.75 g, 56%) as off-white coloured plates; $R_f$ (10% EtOAc/hexanes) 0.32; mp 73-75 ºC; $\delta_H$ ($CDCl_3$): 8.45 (2H,d, *J* 5.5 Hz, 2H); $\delta_C$ ($CDCl_3$): 147.7 (*J* 283 Hz), 139.53, 133.7 (*J* 2.5 Hz), 116.6 (*J* 6.3 Hz); **Note:** compound **7** did not ionize under ESI MS conditions.

*4.2.8. (Z)-1-tert-Butyl 6-methyl 2-(4-bromo-2,6-dinitrophenyl)-3-hydroxyhex-2-enedioate (**20**)*

Sodium hydride (60% dispersion in oil, 5.09 g, 0.127 mol) was washed with dry hexanes then suspended in dry tetrahydrofuran (100 mL) and cooled to 0 ºC. A solution of 1-*tert*-butyl 6-methyl 3-oxohexanedioate[19,20] (15.4 g, 66.9 mmol) in dry tetrahydrofuran (50 mL) was added dropwise over 10 min ($H_2$ gas evolved). After 15 min, a solution of bromide **7** (16.9 g, 63.7 mmol) in dry tetrahydrofuran (100 mL) was added and the mixture was allowed to stir for a further 24 h at room temperature. The reaction mixture was then concentrated under reduced pressure, diluted with dichloromethane (400 mL) and the organic fraction washed with 2 M hydrochloric acid (160 mL). The organic fraction was subject to standard workup to give the crude product as a dark orange solid. This was recrystallised (MeOH) and the mother liquors were further purified by flash chromatography ($CH_2Cl_2$) and recrystallisation (MeOH) to yield the enol **20** (18.7 g, 62%) as pale yellow plates; $R_f$ (15% EtOAc/hexanes) 0.44; mp 137-139 ºC; $\delta_H$ ($CDCl_3$): 13.26 (1H, br s), 8.19 (2H, s), 3.61 (3H, s), 2.56 (2H, t, *J* 7.5 Hz), 2.28 (2H, t, *J* 7.5 Hz), 1.34 (9H, s); $\delta_C$ ($CDCl_3$): 174.2, 172.6, 169.5, 151.5, 130.4, 123.4, 122.5, 95.0, 83.6, 52.0, 29.6, 28.3, 27.9; *m/z* (ESI): 497.0 (M[$^{79}$Br]Na$^+$), 499.0 (M[$^{81}$Br]Na$^+$); HRMS (ESI): M[$^{81}$Br]Na$^+$, found 499.0144. $C_{17}H_{19}BrN_2O_9$ requires 499.0172.

*4.2.9. Methyl 5-(4-bromo-2,6-dinitrophenyl)-4-oxopentanoate (**6**)*

A stirred solution of enol **20** (18.7 g, 39.3 mmol) and 32% hydrochloric acid (30 mL) in methanol (300 mL) was heated to reflux overnight. The mixture was cooled to room temperature and the solid that crystallised from the reaction mixture was collected by filtration. The filtrate was concentrated under reduced pressure, diluted with dichloromethane (200 mL) and then washed with saturated sodium hydrogen carbonate (100 mL). The organic fraction was subject to standard workup to give the crude product, which was recrystallised (MeOH) and combined with the previously isolated material to give the ketone **6** (13.7 g, 93%) as off-white plates; $R_f$ (30% EtOAc/hexanes) 0.60; mp 100-102 ºC; $\delta_H$ ($CDCl_3$): 8.28 (2H, s), 4.31 (2H, s), 3.67 (3H, s), 2.92 (2H, t, *J* 6.6 Hz), 2.64 (2H, t, *J* 6.6 Hz); $\delta_C$ ($CDCl_3$): 201.6, 172.6, 151.4, 131.7, 123.6, 121.8, 52.1, 41.5, 37.1, 27.8; *m/z* (ESI): 396.9 (M[$^{79}$Br]Na$^+$), 298.9 (M[$^{81}$Br]Na$^+$); HRMS (ESI): M[$^{79}$Br]Na$^+$, found 396.9643. $C_{12}H_{11}BrN_2O_7Na$ requires 396.9647.

*4.2.10. Methyl 3-(6-bromo-1-hydroxy-4-nitro-1H-indol-2-yl)propanoate (**21**)*

A suspension of ketone **6** (500 mg, 1.50 mmol) and platinum(IV) oxide (2.5% w/w, 14.0 mg) in methanol (20 mL) was stirred under an atmosphere of hydrogen (balloon). Once the mixture became homogeneous (10-15 min), stirring was continued for a further 5 min until TLC indicated full consumption of the starting material. The reaction mixture was then filtered through Celite® and concentrated under reduced pressure to give a black solid. Purification by flash chromatography (30% EtOAc/hexanes) afforded the *N*-hydroxyindole **21** (270 mg, 62%) as a bright yellow crystalline solid; $R_f$ (40% EtOAc/hexanes) 0.49 (self stains bright yellow, dark orange with ninhydrin); mp 163-165 ºC (dec); $\delta_H$ ($CDCl_3$): 9.73 (1H, br s), 8.21 (1H, d, *J* 1.5 Hz), 7.96 (1H, d, *J* 1.0 Hz), 6.89 (1H, s), 3.68 (3H, s), 3.16 (2H, t, *J* 6.0 Hz), 2.89 (2H, t, *J* 6.0 Hz); $\delta_C$ ($CDCl_3$): 176.4, 141.9, 139.6, 135.7, 120.6, 118.3, 116.5, 112.6, 96.1, 53.0, 35.0, 19.4; *m/z* (ESI): 343.0 (M[$^{79}$Br]H$^+$), 345.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{81}$Br]H$^+$, found 344.9901. $C_{12}H_{12}BrN_2O_5$ requires 344.9930.

*4.2.11. Methyl 3-(4-amino-6-bromo-1H-indol-2-yl)propanoate (**22**)*

To a stirred solution of indole **21** (6.60 g, 19.2 mmol) in 3:1 methanol/acetic acid (300 mL), was added zinc dust (12.6 g, 0.192 mol), portionwise over 30 min. After being stirred overnight, the reaction mixture was concentrated under reduced pressure, diluted with ethyl acetate (100 mL) and filtered. The organic layer was washed with saturated sodium hydrogen carbonate (3 × 30 mL) and treated according to standard workup to give a brown solid. Purification by flash chromatography (0-5% EtOAc/$CH_2Cl_2$) afforded the aminoindole **22** (4.44 g, 78%) as fluffy white crystals; $R_f$ (40% EtOAc/hexanes) 0.49 (dark purple with ninhydrin); mp 137-138 ºC; $\delta_H$ ($CDCl_3$): 8.47 (1H, br s), 6.94 (1H, s), 6.49 (1H, d, *J* 1.5 Hz), 6.10 (1H, d, *J* 1.0 Hz), 3.88 (2H, br s), 3.72 (3H, s), 3.03 (2H, t, *J* 6.5 Hz), 2.71 (2H, t, *J* 6.5 Hz); $\delta_C$ ($CDCl_3$): 174.5, 139.8, 137.5, 136.9, 116.5, 115.6, 107.5, 105.0, 96.5, 52.2, 34.0, 23.1; *m/z* (ESI): 297.0 (M[$^{79}$Br]H$^+$), 299.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 297.0231. $C_{12}H_{14}BrN_2O_2$ requires 297.0239.

*4.2.12. Methyl 3-(6-bromo-4-(pyrrolidine-1-carboxamido)-1H-indol-2-yl)propanoate (**23a**)*

A solution of amine **22** (1.17 g, 3.90 mmol) and pyridine (700 µL, 8.70 mmol) in dry dichloromethane (150 mL) was added dropwise over 2.5 hours to a solution of triphosgene (444 mg, 1.50 mmol) in dry dichloromethane (20 mL) at 0 ºC. Once the addition was complete, a solution of pyrrolidine (0.34 mL, 4.10 mmol) and pyridine (0.7 mL, 8.70 mmol) in dry dichloromethane (5 mL) was added in a single portion. The reaction mixture was stirred for a further 0.5 h at 0 ºC, then washed sequentially with 10% potassium bisulfate (30 mL) and 5% sodium hydrogen carbonate (30 mL). The organic fraction was treated according to standard workup to give the crude product, which was purified by flash chromatography (1-2.5% MeOH/$CH_2Cl_2$) to give the urea **23a** (1.06 g, 68%) as a white solid; $R_f$ (2.5% MeOH/$CH_2Cl_2$) 0.35; mp 166-170 ºC; $\delta_H$ ($CDCl_3$): 8.67 (1H, br s), 7.79 (1H, s), 7.15 (1H, s), 6.28 (1H, br s), 6.08 (1H, s), 3.72 (3H, s), 3.50-3.53 (4H, m), 3.02 (2H, t, *J* 6.5 Hz), 2.70 (2H, t, *J* 6.5 Hz), 1.98-2.01 (4H, m); $\delta_C$ ($CDCl_3$): 174.3, 153.9, 138.2, 137.2, 131.4, 119.5, 115.1, 113.8, 109.2, 96.0, 52.1, 46.0, 33.8, 25.8, 23.2; *m/z* (ESI): 394.1 (M[$^{79}$Br]H$^+$), 396.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{81}$Br]H$^+$, found 396.0742. $C_{17}H_{21}BrN_3O_3$ requires 396.0766.

*4.2.13. Methyl 3-(4-acetamido-6-bromo-1H-indol-2-yl)propanoate (**23b**)*

To a stirred solution of amine **22** (1.77 g, 5.96 mmol) in dry dichloromethane (150 mL), was added triethylamine (870 µL, 6.25 mmol) and acetic anhydride (560 µL, 6.25 mmol). After 4.5 h, the reaction mixture was concentrated under reduced pressure. The crude product was recrystallised from methanol to give the acetamide **23b** (1.60 g, 79%) as a white solid; $R_f$ (2.5% MeOH/$CH_2Cl_2$) 0.27; mp 209-211 ºC; $\delta_H$ (DMSO-$d_6$): 11.13 (1H, br s), 9.62 (1H, s), 7.84 (1H, s), 7.18 (1H, s), 6.51 (1H, s), 3.61 (3H, s), 2.97 (2H, t, *J* 7.5 Hz), 2.75 (2H, t, *J* 7.5 Hz), 2.13 (3H, s); $\delta_C$ (DMSO-$d_6$): 172.4, 168.7, 138.2, 137.1, 131.2, 119.0, 112.6, 112.4, 109.0, 96.7, 51.5, 32.7, 23.9, 23.0; *m/z* (ESI): 339.0 (M[$^{79}$Br]H$^+$), 341.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 339.0337. $C_{14}H_{16}BrN_2O_3$ requires 339.0344.

*4.2.14. 3-(6-Bromo-4-(pyrrolidine-1-carboxamido)-1H-indol-2-yl)propanoic acid (**24a**)*

A mixture of methyl ester **23a** (768 mg, 1.95 mmol) and 15% aqueous sodium hydroxide (10 mL) in methanol (10 mL) was stirred at room temperature overnight. The mixture was acidified with 2 M hydrochloric acid and then extracted with ethyl acetate (3 × 20 mL). The combined organic fractions were treated according to standard workup to give the crude product. Purification by flash chromatography (5% MeOH/$CH_2Cl_2$) afforded the acid **24a** (649 mg, 88%) as a white solid; mp 200-202 ºC; $R_f$ (5% MeOH/$CH_2Cl_2$) 0.21; $\delta_H$ (DMSO-$d_6$): 12.22 (1H, br s), 11.02 (1H, s), 7.73 (1H, s), 7.45 (1H, d, *J* 1.0 Hz), 7.12 (1H, s), 6.33 (1H, s), 3.41-3.43 (4H, m), 2.90 (2H, t, *J* 8.0 Hz), 2.64 (2H, t, *J* 8.0 Hz), 1.85-1.88 (4H, m); $\delta_C$ (DMSO-$d_6$): 173.5, 153.8, 138.2, 137.1, 132.5, 120.2, 113.2, 112.6, 108.0, 97.0, 45.7, 33.1, 25.1, 23.1; *m/z* (ESI): 380.1 (M[$^{79}$Br]H$^+$), 382.1 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 380.0606. $C_{16}H_{19}BrN_3O_3$ requires 380.0610.

*4.2.15. 3-(4-Acetamido-6-bromo-1H-indol-2-yl)propanoic acid (**24b**)*

A mixture of methyl ester **23b** (1.59 g, 4.69 mmol) and 15% aqueous sodium hydroxide (20 mL) in methanol (50 mL) was stirred at room temperature overnight. The mixture was acidified with 2 M hydrochloric acid and the resulting precipitate was collected by filtration and dried *in vacuo* to give the acid **24b** (920 mg, 82%) as an off-white solid; $R_f$ (7.5% MeOH/$CH_2Cl_2$) 0.22; mp 220-224 ºC (dec); $\delta_H$ (DMSO-$d_6$): 12.29 (1H, br s), 11.13 (1H, br s), 9.63 (1H, s), 7.84 (1H, s), 7.18 (1H, s), 6.52 (1H, s), 2.93 (2H, t, *J* 7.5 Hz), 2.65 (2H, t, *J* 7.5 Hz), 2.13 (3H, s); $\delta_C$ (DMSO-$d_6$): 173.5, 168.8, 138.7, 137.2, 131.2, 119.1, 112.6, 112.5, 109.0, 96.6, 33.0, 23.9, 23.1; *m/z* (ESI): 325.0 (M[$^{79}$Br]H$^+$), 327.0 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{79}$Br]H$^+$, found 325.0184. $C_{13}H_{13}BrN_2O_3$ requires 325.0188.

*4.2.16. Preparation of 3-(1H-indol-2-yl)propanamides by EDAC mediated amide coupling*

*4.2.16.1. General procedure A.* A mixture of the acid (1 eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.8 eq.), and 1-hydroxybenzotriazole monohydrate (1.8 eq.) in dry dichloromethane was stirred at room temperature for 0.5 h. The amine (1.1 eq.) was added and the mixture was allowed to stir until TLC indicated full consumption of the starting material. The reaction mixture was washed with water, and treated according to standard workup to afford the crude product.

*4.2.16.2. N-(6-Bromo-2-(3-oxo-3-((2-(piperidin-1-yl)ethyl)amino)propyl)-1H-indol-4-yl)pyrrolidine-1-carboxamide (**5a**)*

Acid **24a** (100 mg, 0.26 mmol) was coupled with 2-(piperidin-1-yl)ethanamine according to General procedure A. Purification by flash chromatography (0-0.5% MeOH/$CH_2Cl_2$ sat. with $NH_3$) followed by recrystallisation ($CH_2Cl_2$) gave the carboxamide **5a** (73 mg, 57%) as white crystals; $R_f$ (2% MeOH/$CH_2Cl_2$ sat. with $NH_3$) 0.20; mp 104-112 ºC; $\delta_H$ (DMSO-$d_6$): 10.97 (1H, br s), 7.76 (1H, t, *J* 5.5 Hz), 7.73 (1H, s), 7.43 (1H, d, *J* 1.5 Hz), 7.11 (1H, d, *J* 1.0 Hz), 6.28 (1H, s), 3.40-3.43 (4H, m), 3.13-3.17 (2H, m (collapses to t, *J* 7.0 Hz upon treatment with $D_2O$)), 2.90 (2H, t, *J* 7.5 Hz), 2.47 (2H, t, *J* 7.5 Hz), 2.25-2.29 (6H, m), 1.85-1.88 (4H, m), 1.43-1.48 (4H, m), 1.34-1.36 (2H, m); $\delta_C$ (DMSO-$d_6$): 170.9, 153.8, 138.6, 137.1, 132.4, 120.3, 113.5, 112.5, 108.0, 97.0, 57.8, 54.1, 45.7, 36.3, 34.8, 25.5, 25.1, 24.0, 23.7; *m/z* (ESI): 490.2 (M[$^{79}$Br]H$^+$), 492.2 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{81}$Br]H$^+$, found 492.1791. $C_{23}H_{33}BrN_5O_2$ requires 492.1818.

*4.2.16.3. 3-(4-Acetamido-6-bromo-1H-indol-2-yl)-N-(2-(piperidin-1-yl)ethyl)propanamide (**5b**)*

Acid **24b** (500 mg, 1.54 mmol) was coupled with 2-(piperidin-1-yl)ethanamine according to General procedure A. Purification by flash chromatography (0.5-2% MeOH/$CH_2Cl_2$ sat. with $NH_3$) gave the carboxamide **5b** (435 mg, 65%) as an beige-coloured

solid; $R_f$ (2.5% MeOH/$CH_2Cl_2$ sat. with $NH_3$) 0.20; mp 84-92 ºC; $\delta_H$ (DMSO-$d_6$): 11.07 (1H, br s), 9.63 (1H, br s), 7.81 (1H, s), 7.75 (1H, t, *J* 5.5 Hz), 7.17 (1H, s), 6.47 (1H, s), 3.12-3.16 (2H, m (collapses to t, *J* 7.0 Hz upon treatment with $D_2O$)), 2.91 (2H, t, *J* 7.5 Hz), 2.47 (2H, t, *J* 7.5 Hz), 2.24-2.28 (6H, m), 2.13 (3H, s), 1.42-1.47 (4H, m), 1.33-1.36 (2H, m); $\delta_C$ (DMSO-$d_6$, 325 K): 170.7, 168.5, 139.0, 137.0, 131.0, 119.3, 112.6, 112.3, 109.0, 96.6, 57.6, 54.1, 36.3, 34.8, 25.4, 23.9, 23.6, 23.5; *m/z* (ESI): 435.1 (M[$^{79}$Br]H$^+$), 437.1 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{81}$Br]H$^+$, found 437.1396. $C_{20}H_{28}BrN_4O_2$ requires 437.1371.

*4.2.16.4. N-(6-Bromo-2-(3-(isobutylamino)-3-oxopropyl)-1H-indol-4-yl)pyrrolidine-1-carboxamide (**5c**)*

Acid **24a** (584 mg, 1.54 mmol) was coupled with 2-methylpropan-1-amine according to General procedure A. Purification by flash chromatography (1-5% MeOH/$CH_2Cl_2$) gave the carboxamide **5c** (540 mg, 81%) as an off-white solid; $R_f$ (2% MeOH/$CH_2Cl_2$) 0.25; mp 104-109 ºC; $\delta_H$ (DMSO-$d_6$): 10.98 (1H, br s), 7.86 (1H, t, *J* 5.5 Hz), 7.72 (1H, s), 7.43 (1H, d, *J* 1.5 Hz), 7.11 (1H, d, *J* 0.5 Hz), 6.28 (1H, s), 3.40-3.43 (4H, m), 2.87-2.92 (4H, m), 2.48 (2H, m (obscured by DMSO peak)), 1.85-1.88 (4H, m), 1.62-1.70 (1H, m), 0.81 (6H, d, *J* 7.0 Hz); $\delta_C$ (DMSO-$d_6$): 171.0, 153.8, 138.7, 137.1, 132.4, 120.4, 113.2, 112.5, 108.1, 96.9, 46.1, 45.7, 34.7, 28.1, 25.1, 23.7, 20.1; *m/z* (ESI): 435.1 (M[$^{79}$Br]H$^+$), 437.1 (M[$^{81}$Br]H$^+$); HRMS (ESI): M[$^{81}$Br]H$^+$, found 437.1371. $C_{20}H_{28}BrN_4O_2$ requires 437.1396.

*4.2.17. N-(2-(3-Oxo-3-((2-(piperidin-1-yl)ethyl)amino)propyl)-6-(6-ureido-1H-indazol-4-yl)-1H-indol-4-yl)pyrrolidine-1-carboxamide (**3a**)*

A mixture of bromide **18** (100 mg, 0.39 mmol), (1,1'-bis(diphenylphosphino)ferrocene)palladium(II) dichloride (14.0 mg, 5 mol %), bis(pinacolato)diboron (139 mg, 0.55 mmol) and potassium acetate (115 mg, 1.18 mmol) in methanol (5 mL) was degassed under high vacuum, purged with argon, and then heated to reflux for 16 h. A solution of caesium carbonate (345 mg, 1.06 mmol) in water (0.5 mL) was added and the mixture was stirred at reflux for a further 3 h then concentrated under reduced pressure. Bromide **5a** (100 mg, 0.52 mmol), (1,1'-bis(diphenylphosphino)ferrocene)palladium(II) dichloride (14.0 mg, 5 mol %) and a 4:1 mixture of 1,4-dioxane/water (5 mL) were added to the residue. The mixture was degassed under high vacuum, purged with argon, and then heated to reflux for 4 h. The reaction mixture was cooled to room temperature, filtered and then concentrated under reduced pressure to give the crude product. Flash chromatography (5-10% MeOH/$CH_2Cl_2$ sat. with $NH_3$) gave an off-white solid (29.0 mg, 24%), which was further purified by preparative reverse phase HPLC (5-65% MeCN/10 mM aq. $NH_4CO_3$) to give the title compound **3a** as a fluffy, white solid (5.6 mg, 5%); $R_f$ (10% MeOH/$CH_2Cl_2$ sat. with $NH_3$): 0.17; mp > 300 ºC; $\delta_H$ (DMSO-$d_6$): 12.80 (1H, br s), 10.98 (1H, br s), 8.82 (1H, s), 8.13 (1H, s), 7.87 (1H, s), 7.82 (1H, t, *J* 5.5 Hz), 7.78 (1H, s), 7.66 (1H, d, *J* 1.0 Hz), 7.27 (1H, s), 6.98 (1H, d, *J* 1.5 Hz), 6.31 (1H, s), 5.89 (2H, br s), 3.36-3.47 (4H, m), 3.15-3.19 (2H, m (collapses to t, *J* 7.0 Hz upon treatment with $D_2O$)), 2.95 (2H, t, *J* 7.5 Hz), 2.53 (2H, m (obscured by DMSO peak)), 2.32-2.26 (6H, m) 1.88-1.90 (4H, m), 1.43-1.48 (4H, m), 1.33-1.36 (2H, m); $\delta_C$ (DMSO-$d_6$): 171.1, 156.2, 154.3, 141.6, 139.1, 139.0, 137.1, 135.6, 133.1, 131.5, 131.3, 121.7, 116.7, 112.0, 111.7, 105.1, 96.9, 95.0, 57.8, 54.1, 45.7, 36.3, 35.0, 25.5, 25.1, 24.0, 23.9; *m/z* (ESI): 586.3 (MH$^+$); HRMS (ESI): MH$^+$, found 586.3252. $C_{31}H_{40}N_9O_3$ requires 586.3254.

*4.2.18. Methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-6-carboxylate (**4b**)*

A mixture of bromide **13** (850 mg, 3.33 mmol), (1,1'-bis(diphenylphosphino)ferrocene)palladium(II) dichloride (73.0 mg, 3 mol %), bis(pinacolato)diboron (1.18 g, 4.62 mmol) and potassium acetate (981 mg, 9.99 mmol) in methanol (30 mL) was degassed under high vacuum, purged with argon, and then heated to reflux for 16 h. The reaction mixture was concentrated under reduced pressure, diluted with dichloromethane (30 mL) and washed with water (50 mL). The organic fraction was treated according to standard workup to give the crude product. Purification by flash chromatography (20% EtOAc/hexanes) gave an inseparable 9:1 mixture of the dioxaborolane **4b** (776 mg, 77%) and methyl 1*H*-indazole-6-carboxylate (50 mg, 9%) as a dark orange crystalline solid; $R_f$ (25% EtOAc/hexanes) 0.30; mp 146-147 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.51 (1H, br s), 8.28 (2H, s), 8.07 (1H, s), 3.91 (3H, s), 1.36 (12H, s); *m/z* (ESI): 303.1 (MH$^+$); This mixture was used in subsequent reactions without further purification.

*4.2.19. Monoligated palladium catalyzed Suzuki cross-coupling*

*4.2.19.1. General procedure B.* Aryl bromide (1 eq.), 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (10 mol %), tris(dibenzylideneacetone)dipalladium(0) (5 mol %), sodium carbonate (2.0 eq.) and dioxaborolane **4b** (1.5 eq.) were suspended in a 4:1 mixture of 1,4-dioxane/water (5 mL). The mixture was degassed under high vacuum, purged with argon, and then heated to reflux for 2-4 h. Upon completion, the mixture was cooled to room temperature, filtered and then concentrated under reduced pressure to give the crude product.

*4.2.19.2. Methyl 4-(2-(3-oxo-3-((2-(piperidin-1-yl)ethyl)amino)propyl)-4-(pyrrolidine-1-carboxamido)-1H-indol-6-yl)-1H-indazole-6-carboxylate (**3b**)*

Bromide **5a** (100 mg, 0.20 mmol) was treated according to General procedure B. Purification by flash chromatography (2-5% MeOH/$CH_2Cl_2$ sat. with $NH_3$) yielded the title compound **3b** (67 mg, 56%) as a white solid; $R_f$ (5% MeOH/$CH_2Cl_2$ sat. with $NH_3$): 0.40; mp 136-148 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.55 (1H, br s), 10.98 (1H, d, *J* 1.5 Hz), 8.41 (1H, s), 8.09 (1H, s), 7.83 (1H, s), 7.80 (1H, t, *J* 5.5 Hz), 7.78 (1H, d, *J* 1.0 Hz), 7.70 (1H, d, *J* 1.0 Hz), 7.35 (1H, s), 6.33 (1H, s), 3.93 (3H, s), 3.45-3.48 (4H, m), 3.15-3.19 (2H, m (collapses to t, *J* 7.0 Hz upon treatment with $D_2O$)), 2.97 (2H, t, *J* 7.5 Hz), 2.53 (2H, m (obscured by DMSO peak)), 2.27-2.30 (6H, m) 1.88-1.91 (4H, m), 1.44-1.48 (4H, m), 1.34-1.36 (2H, m); $\delta_C$ (DMSO-$d_6$): 171.2, 166.8, 154.4, 140.3, 139.4, 137.2, 136.2, 133.7, 131.5, 130.7, 127.7, 123.9, 122.0, 118.4, 112.1, 110.2, 105.5, 97.2, 57.9, 54.1, 52.4, 45.8, 36.4, 35.0, 25.5, 25.2, 24.1, 23.9; *m/z* (ESI): 586.3 (MH$^+$); HRMS (ESI): MH$^+$, found 586.3142. $C_{32}H_{40}N_7O_4$ requires 586.3142.

*4.2.19.3. Methyl 4-(2-(3-(isobutylamino)-3-oxopropyl)-4-(pyrrolidine-1-carboxamido)-1H-indol-6-yl)-1H-indazole-6-carboxylate (**3c**)*

Bromide **5c** (100 mg, 0.23 mmol) was treated according to General procedure B. Purification by flash chromatography (1-3% MeOH/$CH_2Cl_2$ sat. with $NH_3$) yielded the title compound **3c** (86 mg, 70%) as an off-white solid; $R_f$ (2% MeOH/$CH_2Cl_2$ sat. with $NH_3$): 0.21; mp 149-162 ºC (dec); $\delta_H$ (DMSO-$d_6$): 13.56 (1H, br s), 10.99 (1H, br s), 8.41 (1H, s), 8.09 (1H, s), 7.90 (1H, t, *J* 5.5 Hz), 7.82 (1H, s), 7.76 (1H, d, *J* 1.0 Hz), 7.69 (1H, d, *J* 1.5 Hz), 7.35 (1H, s), 6.34 (1H, s), 3.93 (3H, s), 3.44-3.47 (4H, m), 2.98 (2H, t, *J* 7.5 Hz), 2.90 (2H, t, *J* 6.5 Hz), 2.54 (2H, t, *J* 7.5 Hz), 1.88-1.91 (4H, m), 1.64-1.72 (1H, m), 0.83 (6H, d, *J* 6.5

Hz); $\delta_C$ (DMSO-$d_6$): 171.1, 166.7, 154.3, 140.2, 139.4, 137.1, 136.1, 133.5, 131.5, 130.6, 127.5, 123.8, 122.0, 118.3, 111.9, 110.0, 105.4, 97.0, 52.3, 46.1, 45.7, 34.9, 28.1, 25.1, 23.9, 20.1; *m/z* (ESI): 531.3 ($MH^+$); HRMS (ESI): $MH^+$, found 531.2718. $C_{29}H_{35}N_6O_4$ requires 531.2720.

*4.2.19.4. Methyl 4-(4-acetamido-2-(3-oxo-3-((2-(piperidin-1-yl)ethyl)amino)propyl)-1H-indol-6-yl)-1HI-indazole-6-carboxylate (**3d**)*

Bromide **5b** (100 mg, 0.23 mmol) was treated according to General procedure B. Purification by flash chromatography (2.5-5% MeOH/$CH_2Cl_2$ sat. with $NH_3$) yielded the title compound **3d** (82 mg, 67%) as an off-white solid; $R_f$ (2.5% MeOH/$CH_2Cl_2$ sat. with $NH_3$): 0.11; mp 147-154 ºC; $\delta_H$ (DMSO-$d_6$): 13.56 (1H, br s), 11.07 (1H, s), 9.71 (1H, s), 8.36 (1H, s), 8.10 (1H, s), 8.07 (1H, s), 7.77-7.79 (2H, m), 7.40 (1H, s), 6.50 (1H, s), 3.93 (3H, s), 3.15-3.19 (2H, m), 2.98 (2H, t, *J* 7.5 Hz), 2.53 (2H, m (obscured by DMSO peak)), 2.26-2.29 (6H, m), 2.16 (3H, s), 1.43-1.47 (4H, m), 1.32-1.35 (2H, m); $\delta_C$ (DMSO-$d_6$): 171.0, 168.6, 166.7, 140.2, 139.7, 137.2, 136.0, 133.4, 130.7, 130.3, 127.6, 123.8, 120.7, 118.5, 111.0, 110.2, 106.3, 96.7, 57.85, 54.1, 52.4, 36.3, 35.0, 25.5, 24.0, 23.9 (2 overlapping signals); *m/z* (ESI): 531.3 ($MH^+$); HRMS (ESI): $MH^+$, found 531.2722. $C_{29}H_{35}N_6O_4$ requires 531.2720.

*4.2.19.5. Methyl 4-(2-(3-methoxy-3-oxopropyl)-4-(pyrrolidine-1-carboxamido)-1H-indol-6-yl)-1H-indazole-6-carboxylate (**3e**)*

Bromide **23a** (100 mg, 0.23 mmol) was treated according to General procedure B. Purification by flash chromatography (2.5% MeOH/$CH_2Cl_2$) yielded the title compound **3e** (36 mg, 44%) as a white solid; $R_f$ (2.5% MeOH/$CH_2Cl_2$): 0.20; mp 142-155 ºC; $\delta_H$ (DMSO-$d_6$): 13.52 (1H, br s), 11.02 (1H, d, *J* 1.5 Hz), 8.41 (1H, s), 8.10 (1H, s), 7.80 (1H, s), 7.79 (1H, d, *J* 1.0 Hz), 7.73 (1H, d, *J* 1.0 Hz), 7.36 (1H. s), 6.39 (1H, s), 3.93 (3H, s), 3.64 (3H, s), 3.46-3.49 (4H, m), 3.04 (2H, t, *J* 7.5 Hz), 2.79 (2H, t, *J* 7.5 Hz), 1.88-1.91 (4H, m); $\delta_C$ (DMSO-$d_6$): 172.4, 166.6, 154.2, 140.1, 138.3, 137.2, 136.0, 133.5, 131.5, 130.8, 127.5, 123.8, 121.7, 118.3, 111.9, 110.0, 105.3, 97.1, 52.2, 51.4, 45.7, 32.9, 25.0, 23.1; *m/z* (ESI): 490.2 ($MH^+$); HRMS (ESI): $MH^+$, found 490.2093. $C_{26}H_{28}N_5O_5$ requires 490.2091.

**Acknowledgements**

The authors would like to thank Dr. Sebastian Marcuccio of Advanced Molecular Technologies Pt. Ltd. (AMT) for assistance with cross-coupling chemistry and Dr. Les Deady (LIMS) for helpful synthesis discussions. We thank Dr. Jason Dang (MIPS) for HRMS analysis. MB is grateful to La Trobe University for financial support through the provision of a La Trobe University Postgraduate Scholarship and the LIMS Writing-Up Award.

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## Graphical Abstract

**Synthesis of substituted 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential PDK1 inhibitors**

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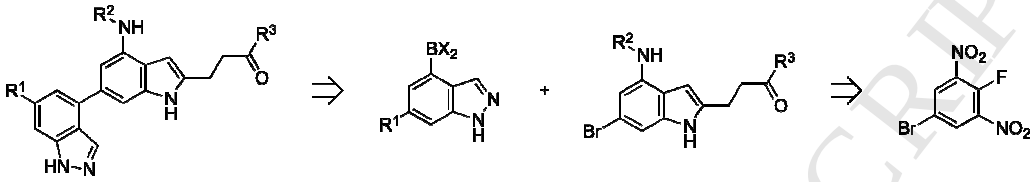

**Supplementary Information**

# Synthesis of substituted 4-(1*H*-indol-6-yl)-1*H*-indazoles as potential PDK1 inhibitors

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## Computational Methodology

### Molecular modelling protocol

A series of ligands were designed by hybridisation/fragmentation of known PDK1 inhibitors to provide novel structures. These structures were then evaluated *in silico* by performing molecular dynamics (MD) simulations followed by free binding energy calculations using the AMBER software package[1]. It should be noted that generation of the protein/ligand complexes is intended to generate an appropriate starting geometry for MD simulation rather than a fully optimised, low-energy structure. An optimised structure will be achieved following minimisation and equilibration steps in the MD protocol.

### Ligand setup and parameterisation

Initial ligand structures were generated using the Maestro module of Schrödinger[2] and exported as pdb files. Hydrogen atoms and connectivity information were added using the LEaP module of AMBER and double bonds were added manually. Ligand atomic partial charges were obtained by performing quantum chemical calculations using Gaussian 03[3]. Single point energy calculations were carried out at the B3LYP level of theory using a ccp-VTZ basis set. Solvent effects were included by a polarisable continuum model (PCM)[4,5] self-consistent reaction field (SCRF) method with an ether-type solvent (diethyl ether $\varepsilon = 4.24$). The resulting electrostatic potentials (ESP) generated from the quantum calculations were then transformed into atomic charges in the antechamber module of AMBER using the restrained ESP (RESP) methodology[6]. Force field parameters were automatically generated in antechamber and these were imported into the General AMBER Force Field (GAFF)[7].

### Preparation of complexes

The structure of PDK1 in complex with ATP (PDB code: 2BIY)[8] was selected for use in our modelling as it was the most complete structure available at the time and was of high resolution. The crystallographic data was edited to remove water, glycerol and sulfate ions. The ATP molecule was also removed to give a PDK1 structure with a vacant ATP binding site. Missing protein side chains and hydrogen atoms added manually in LEaP using the ff03 force field[9] to provide an 'empty' PDK1 template. Maestro was used to manually dock the ligand molecules into the enzyme active site of the 'empty' PDK1 structure using a superimposition method. The PDK1/ligand complexes were charge neutralised by the addition of counterions and solvated by a TIP3P solvent box with a minimum solute wall distance of 10 Å.

### MD simulations

Five successive minimization steps were performed: (i) 5000-step minimisation [1000 step steepest-descent (SD) then 4000-step conjugate gradient (CG)] with protein/ligand system restrained to relax solvent and counterion molecules, (ii) three rounds of 1000-step minimisation [100 with SD and 900 with CG] with decreasing restraints

of 15, 10, 5 kcal/mol applied to the protein C-α backbone atoms and (iii) 1000-step minimisation [100 with SD and 900 with CG] with all restraints removed. The system was then heated to 300 K using Langevin dynamics[10] in a slow, stepwise fashion (30 K every 2500 steps). Equilibration was then performed in the NPT ensemble for 50,000 steps (a total of 100 ps with a 2 fs timeframe) with all restraints removed. The SHAKE algorithm[11] was used to constrain bond lengths for bonds involving hydrogen with a cutoff of 12 Å for non-bonded interactions. Pressure was regulated by way of isotropic position scaling and temperature was held constant at 300 K with regulation by Langevin dynamics. A production phase of 5 ns was completed in five 1ns blocks, using the same conditions as the equilibration phase. Post-production analysis was performed to ensure the system structure had remained realistic and that the equilibrium structure had not changed drastically. This was done by viewing the output trajectory file in VMD[12] to ensure the protein remained folded and also by plotting the physical parameters to ensure that they remained stable.

**Free binding energy calculations**

After the product dynamics, the resulting MD trajectory was used to perform free energy calculations. This was done using the single trajectory approach, with all of the snapshots coming from the one trajectory rather than separate trajectories for the complex, receptor and the ligand. Snapshots were generated from the MD trajectories at 10 ps intervals to produce a series of uncorrelated structures for each of the complex, receptor and ligand. The free binding energies were then calculated using the MM_PBSA script in AMBER.

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